

WHAT IS CLAIMED IS:

~~1.~~ A method for producing one or more transposable element component with a desired property, the method comprising:

- 5 i) providing a population of polynucleotide segments comprising at least one transposable element or subportion of a transposable element;
- ii) recombining the polynucleotide segments one or more times, thereby producing a library of recombinant transposable element components;
- iii) identifying at least one recombinant transposable element component with a desired property;
- 10 iv) optionally repeating steps (i) through (iii) at least one additional time.

2. The method of claim 1, further comprising recovering the transposable element or a subportion thereof by a polymerase chain reaction (PCR), ligase chain reaction (LCR), Q $\beta$ -replicase amplification, NASBA or cloning.

3. The method of claim 1, comprising providing a population of  
15 polynucleotide segments comprising at least one component of a transposon or insertion sequence (IS) element or a subportion of a component of a transposon or IS element.

4. The method of claim 3, wherein the at least one component of a transposable element comprises an inverted repeat or a transposase of a transposon or an IS element.

20 5. The method of claim 3, wherein the transposon or IS element comprises a mini-transposon or a mini-IS element.

6. The method of claim 1, wherein at least one polynucleotide segment comprises a transposable element or a subportion of a transposable element of a bacterium, a fungus, a plant or an animal.

25 7. The method of claim 1, wherein the transposable element comprises a Class I or a Class II transposable element.

8. The method of claim 7, wherein the Class I transposable element comprises a retrotransposon, a retroposon or a SINE-like element.

9. The method of claim 8, wherein the Class I transposable element comprises a *Ty-1* family transposon, a *Copia* family transposon, or a *gypsy* family transposon.

10. The method of claim 7, wherein the Class II transposable element comprises a *Fot1/Pogo* family transposon, a *Tc1/Mariner* family transposon,

11. The method of claim 7, wherein the transposable element is selected from the group consisting of TN3, TN5, TN10, TN917, ISS1, TN5990, Ty1, Ty2, Ty3, and mariner.

12. The method of claim 1, comprising recombining the polynucleotide segments in vitro, in vivo, or in silico.

13. The method of claim 1, wherein the desired property is selected from one or more of altered specificity of integration, host adaptation, altered cofactor specificity, increased or decreased recombinase activity, increased or decreased transposase activity, increased or decreased recombinase specificity, increased or decreased transposase specificity, increased or decreased size of exogenous DNA transposed, increased or decreased copy number, increased or decreased efficiency of transposition, increased or decreased preference for episomal targeting, increased or decreased preference for chromosomal targeting, increased efficiency of integration into non-supercoiled DNA, and increased efficiency of in vitro transposition.

14. The method of claim 1, wherein the identifying of step (iii) comprises screening or selecting at least one transposable element with a desired property.

15. The method of claim 14, comprising identifying at least one transposable element that mediates transposition in vitro with greater efficiency when compared to a parental transposable element, the method comprising:

providing a plurality of in vitro transposition reactions, which in vitro transposition reactions comprise:

- (a) a transposase;
- (b) a donor polynucleotide comprising at least one inverted repeat; and

5 (c) a target polynucleotide

incubating the plurality of in vitro transposition reactions under conditions permissive for in vitro transposition; and

identifying at least one in vitro transposition reaction that occurs with greater efficiency than an in vitro transposition reaction mediated by a parental transposable element.

10                   **16.** The method of claim 15, comprising providing in vitro transposition reactions comprising transposomes, which transposomes comprise the transposase and the donor polynucleotide.

15                   **17.** The method of claim 14, comprising identifying at least one transposable element that transposes with increased efficiency in a specified host cell when compared with a wild type transposable element, the method comprising:

- (a) introducing a plurality of transposable elements, which transposable elements differ by at least one nucleotide, into a population of host cells;
- (b) selecting at least one host cell that has integrated the transposable element into a chromosome or episome.

20                   **18.** The method of claim 17, the transposable element comprising in the direction of transcription (a) a polynucleotide comprising a transcription regulatory sequence; (b) a 5' splice donor site; (c) a first inverted repeat; (d) a 3' splice acceptor site; (e) a polynucleotide encoding a transposase; (f) a polynucleotide encoding a selectable marker; and (g) a second inverted repeat.

25                   **19.** The method of claim 18, which transposable element transiently expresses the transposase.

20. The method of claim 17, comprising selecting at least one host cell that expresses a sufficient level of a selectable marker encoded by the transposable element.

5 21. The method of claim 17, further comprising recovering the transposable element.

22. The method of claim 17, the population of host cells comprising mammalian cells.

10 23. The method of claim 17, wherein the transposable element comprises a *Mariner* transposase, and wherein the inverted repeats comprise *Mariner* inverted repeats.

24. The method of claim 23, wherein the *Mariner* transposase comprises a Himar1 transposase.

25. The method of claim 17, wherein the selectable marker comprises drug resistance.

15 26. The method of claim 25, wherein the antibiotic resistance is selected from among neomycin resistance, kanamycin resistance.

27. The method of claim 1, wherein the transposable element comprises a recombinant vector.

20 28. The method of claim 27, wherein the recombinant vector is a delivery vector comprising (a) an origin of replication active in at least one cloning host; (b) a conditional origin of replication active in at least one target cell; (c) at least one screenable or selectable marker; (d) a mini-transposon comprising a first inverted repeat and a second inverted repeat, which inverted repeats flank a multicloning site (MCS); and (e) a transposase operably linked to a promoter active in at least one target cell.

25 29. The method of claim 28, wherein the transposase is in close proximity to at least one end of the minitransposon.

**30.** The recombinant delivery vector of claim 28.

**31.** The recombinant delivery vector of claim 30, the origin of replication (a) comprising an origin of replication selected from among a ColE1 origin, a pACYC origin, a p15A origin, an RK4 origin, an RK6 origin, a pCM595 origin, a pSa origin, a pUB110 origin, a pE194 origin, a pG+ origin, a 2 micron circle origin, and an artificial chromosome origin.

**32.** The recombinant delivery vector of claim 30, the conditional origin of replication (b) comprising a temperature sensitive origin of replication selected from among a gram negative origin, a pSA3 origin, a pE194tm origin, and a pG+tm origin.

**33.** The recombinant delivery vector of claim 30, the at least one selectable or screenable marker (c) comprising antibiotic resistance, conferred prototrophy, or toxicity resistance.

**34.** The recombinant delivery vector of claim 31, wherein the antibiotic resistance marker comprises kanamycin resistance, ampicillin resistance, macolide-lincosamine-streptogramin (MLS) resistance, apramycin resistance, spiramycin resistance, hygromycin resistance, chloramphenicol resistance, or tetracycline resistance.

**35.** The recombinant delivery vector of claim 30, wherein the mini-transposon (d) is derived from a transposon or insertion sequence of table 1.

**36.** The recombinant delivery vector of claim 30, the transposase (e) comprising a naturally occurring transposase or a transposase derived by one or more directed evolution method.

**37.** The recombinant delivery vector of claim 36, wherein the promoter is selected from an endogenous promoter of a target cell.

**38.** The recombinant delivery vector of claim 30, comprising in the order of transcription: a polynucleotide encoding a transposase operably linked to a promoter functional in a target cell, a mini IS element, which mini-IS element comprises a first IS inverted repeat and a second IS inverted repeat, which first and second IS inverted

repeats flank a multicloning site, a first origin of replication functional in cloning host, a first selectable marker, a second selectable marker, and a second origin of replication, which origin of replication is temperature sensitive.

39. The recombinant delivery vector of claim 38, wherein the  
5 transposase comprises: a transposon or IS element int encoding sequence and a transposon or IS element xis encoding sequence.

40. The recombinant delivery vector of claim 38, wherein the first or  
second selectable marker comprises a drug resistance marker selected from: ampicillin  
resistance, kanamycin resistance, chloramphenicol resistance, neomycin resistance,  
10 tetracycline resistance, erythromycin resistance and G418 resistance.

41. The recombinant delivery vector of claim 38, wherein the first and  
second selectable markers comprise two alternative markers selected from: ampicillin  
resistance, kanamycin resistance, chloramphenicol resistance, neomycin resistance,  
tetracycline resistance, erythromycin resistance and G418 resistance.

42. The recombinant delivery vector of claim 38, wherein the second  
15 origin of replication comprises a thermosensitive replicon of pG+.

43. The recombinant delivery vector of claim 38, wherein the vector  
comprises a pTNWGS vector.

44. A transposable element with a desired property produced by the  
20 method of claim 1.

45. The transposable element of claim 44, wherein the desired property  
is selected from one or more of altered specificity of integration, host adaptation,  
increased or decreased recombinase activity, increased or decreased transposase activity,  
increased or decreased recombinase specificity, increased or decreased transposase  
25 specificity, increased or decreased size of exogenous DNA transposed, increased or  
decreased copy number, increased or decreased efficiency of transposition, increased or  
decreased preference for episomal targeting, increased or decreased preference for

chromosomal targeting, increased efficiency of integration into non-supercoiled DNA, and increased efficiency of in vitro transposition.

**46.** The transposable element of claim 44, which transposable element catalyzes in vitro transposition more efficiently than a parental transposable element.

5                   **47.** The transposable element of claim 44, which transposable element integrates into a specified host cell with increased efficiency when compared to a wild type transposable element.

**48.** A component of a transposable element with a desired property produced by the method of claim 1.

10                   **49.** The transposable element component of claim 48, wherein the component comprises a transposase, a recombinase or an integrase.

**50.** The transposable element component of claim 49, comprising a transposase that catalyzes in vitro transposition more efficiently than a parental transposase.

15                   **51.** The transposable element component of claim 48, wherein the component comprises an inverted repeat.

~~**52.**~~ A method for producing a transposase that efficiently catalyzes in vitro transposition, the method comprising:

- 20                   i) providing a population of polynucleotide segments encoding at least one transposase or subportion of a transposase;
- ii) recombining the polynucleotide segments one or more times, thereby producing a library of recombinant polynucleotides encoding variant transposases;
- iii) identifying at least one recombinant polynucleotide encoding a transposase that efficiently catalyzes in vitro transposition.

25                   **53.** The method of claim 52, comprising identifying the at least one recombinant polynucleotide encoding a transposase that efficiently catalyzes in vitro transposition by:

- a) providing a plurality of in vitro transposition reactions, which in vitro transposition reactions comprise a transposase encoded by the recombinant polynucleotide, a donor polynucleotide comprising at least one inverted repeat, and a target polynucleotide;
- b) incubating the plurality of in vitro transposition reactions under conditions permissive for in vitro transposition; and
- c) identifying at least one in vitro transposition reaction that occurs with greater efficiency than an in vitro transposition reaction mediated by a parental transposase.

**54.** A transposase produced by the method of claim 52.

**55.** The transposase of claim 54, wherein the transposase is selected from among transposases derived by a directed evolution process from at least one transposase of TN5, TN10, TN917, ISS1, TN5990, Ty1, Ty2, Ty3, or mariner.

**56.** A reaction mix or a cell comprising the transposase of claim 54.

~~**57.**~~ A method for generating diversity in a population of nucleic acids, the method comprising: contacting at least one recombinant transposable element or recombinant transposable element component, and a plurality of subject nucleic acids under conditions permissive for transposition.

**58.** The method of claim 57, wherein the recombinant transposable element or recombinant transposable element component is produced by one or more diversity generating procedure.

**59.** The method of claim 57, wherein the recombinant transposable element or recombinant transposable element component is produced by recursive recombination.

**60.** The method of claim 57, further comprising identifying at least one altered subject nucleic acid.

**61.** The method of claim 57, comprising contacting the recombinant transposable element or recombinant transposable element component and the subject nucleic acids in vivo.

**62.** The method of claim 61, wherein the recombinant transposable element component comprises a recombinant transposase.

**63.** The method of claim 62, comprising introducing a transposome, which transposome comprises the recombinant transposase bound to a donor nucleic acid,  
5 which donor nucleic acid comprises sequences recognized by the recombinant transposase, into a cell, thereby contacting the recombinant transposable element component and the subject nucleic acids.

**64.** The method of claim 63, comprising introducing the transposome into the cell by electroporation.

10 **65.** The method of claim 57, comprising contacting the transposable element or transposable element component and the subject nucleic acids in vitro.

**66.** The method of claim 65, wherein the recombinant transposable element component comprises a recombinant transposase.

15 **67.** The method of claim 65, comprising contacting the subject nucleic acids with a transposome, which transposome comprises the shuffled transposase bound to a donor nucleic acid, which donor nucleic acid comprises sequences recognized by the shuffled transposase, in an acellular reaction mix.

~~**68.**~~ A method for generating diversity in a population of nucleic acids, the method comprising:

- 20 i) providing a plurality of transposomes, which transposomes comprise a library of donor nucleic acids, and a population of acceptor nucleic acids in vitro;  
ii) recombining the donor nucleic acids and the acceptor nucleic acids to produce a library of recombinant nucleic acids.

25 **69.** The method of claim 68, comprising recombining the donor nucleic acids and the acceptor nucleic acids in the presence of magnesium ions.

**70.** The method of claim 68, comprising providing the transposome by combining a plurality of donor nucleic acid molecules, which donor nucleic acid

molecules comprise transposable element recognition sequences and a plurality of transposase molecules, which transposase molecules bind the transposable element recognition sequences.

5       **71.** The method of claim 70, wherein the donor nucleic acids comprising transposable element recognition sequences are produced by cloning genomic DNA fragments into a mini-transposon or mini-insertion sequence.

**72.** The method of claim 71, wherein the genomic DNA fragments are restriction enzyme fragments.

10       **73.** The method of claim 71, wherein the mini-transposon comprises a Tn5 mini-transposon.

**74.** The method of claim 71, wherein the mini-transposon comprises a mariner transposon.

**75.** The method of claim 68, wherein one or more of the donor or acceptor nucleic acids are derived from a plurality of organisms.

15       **76.** The method of claim 68, further providing at least one population of additional nucleic acids.

20       **77.** The method of claim 76, the population of additional nucleic acids comprising one or more of a promoter, a regulatory element, a terminator sequence, an antiterminator sequence, a sequence comprising a start codon, a sequence comprising a stop codon, a library of recombinant genes, a gene of interest, or an IS element.

**78.** The method of claim 68, further comprising repeating the recombination of steps i) and ii) by providing transposomes comprising the library of recombinant nucleic acids or a subportion thereof.

25       **79.** The method of claim 68, further comprising, introducing the library of recombinant nucleic acids or a subportion thereof into a population of cells and identifying at least one cell with a desired property.

**80.** The method of claim 79, comprising introducing the library of recombinant nucleic acids or a subportion thereof into the population of cells by a delivery method comprising natural competence, conjugation, transformation, electroporation, or infection with bacteriophage.

5                    ~~81.~~ A method for identifying a chromosomal locus, which chromosomal locus exhibits a desired level of gene expression, the method comprising:

- 10                    i)        transfecting a plurality of host cells expressing a transposase with a vector comprising, in the direction of transcription: (a) a first inverted repeat; (b) a promoter; (c) a site specific recombinase recognition site; (d) a polynucleotide encoding a first screenable or selectable marker; (e) a polynucleotide encoding a second screenable or selectable marker; and (f) a second inverted repeat;
- 15                    ii)       identifying at least one host cell that expresses a sufficient level of at least one selectable marker, which selectable marker is encoded by the first or second visible or selectable marker, to survive selection, thereby identifying at least one host cell that has integrated the vector into a chromosome; and
- 20                    iii)      identifying at least one host cell expressing at least one screenable or selectable marker at a desired level, thereby identifying a chromosomal locus exhibiting a desired level of gene expression.

25                    **82.** The method of claim 81, wherein the vector further comprises a polynucleotide encoding the transposase operably linked to a promoter active in the host cells.

**83.** The method of claim 81, further comprising integrating a polynucleotide sequence of interest into the identified chromosomal locus to generate at least one integrant.

30                    **84.** The method of claim 82, further comprising identifying at least one integrant with a desired level of expression.

**85.** The method of claim 81, wherein the inverted repeats comprise transposable element inverted repeats.

**86.** The method of claim 85, wherein the inverted repeats comprise *Mariner* inverted repeats.

**87.** The method of claim 81, wherein the site specific recombinase recognition site comprises a loxP site.

5                   **88.** The method of claim 81, wherein the promoter comprises a cytomegalovirus (CMV) promoter.

**89.** The method of claim 81, wherein the first or second screenable or selectable marker is a selectable marker selected from among: antibiotic resistance, herbicide resistance, neomycin resistance, kanamycin resistance.

10                   **90.** The method of claim 81, wherein the first or second screenable or selectable marker is a visible marker selected from among: green fluorescent protein (GFP), luciferase,  $\beta$ -galactosidase,  $\beta$ -glucuronidase, alkaline phosphatase.

15                   **91.** The method of claim 81, wherein the first screenable or selectable marker comprises a visible marker and the second screenable or selectable marker comprises a selectable marker.

**92.** The method of claim 91, wherein the visible marker is GFP and the selectable marker is neomycin resistance.

**93.** The method of claim 81, the plurality of cells comprising bacterial, fungal, animal or plant cells.

20                   **94.** The method of claim 81, wherein the transposase is encoded by a chromosomal sequence.

**95.** The method of claim 81, wherein the transposase is encoded by a polynucleotide comprising an additional vector.

25                   **96.** The method of claim 95, wherein the additional vector comprises an episomal vector.

**97.** The method of claim 95, wherein the vector comprises a chromosomally integrated vector.

**98.** The method of claim 95, comprising expressing the transposase transiently.

5           **99.** The method of claim 98, comprising expressing the transposase inducibly.

**100.** The method of claim 81, comprising expressing a *Mariner* transposase.

10           **101.** The method of claim 100, wherein the transposase comprises an artificially evolved transposase, which artificially evolved transposase has at least one property which differs from a parental transposase from which it is derived by directed evolution.

15           **102.** The method of claim 101, wherein the at least one property which differs from the parental transposase is selected from among: sequence specificity, activity level, species selectivity, allostery, and control.

~~**103.**~~ A vector comprising (a) a first inverted repeat; (b) a promoter; (c) a site specific recombinase recognition site; (d) a polynucleotide encoding a first screenable or selectable marker; (e) a polynucleotide encoding a second screenable or selectable marker; and (f) a second inverted repeat.

20           **104.** The vector of claim 103, wherein the inverted repeats comprise transposable element inverted repeats.

**105.** The vector of claim 104, wherein the inverted repeats comprise *Mariner* inverted repeats.

25           **106.** The vector of claim 103, wherein the site specific recombinase recognition site is a loxP site.

107. The vector of claim 103, wherein the promoter comprises a cytomegalovirus (CMV) promoter.

108. The vector of claim 103, wherein the first or second screenable or selectable marker is a selectable marker selected from among: antibiotic resistance,  
5 herbicide resistance, neomycin resistance, kanamycin resistance.

109. The vector of claim 103, wherein the first or second screenable or selectable marker is a visible marker selected from among: green fluorescent protein (GFP), luciferase,  $\beta$ -galactosidase,  $\beta$ -glucuronidase, alkaline phosphatase.

110. The vector of claim 103, wherein the first screenable or selectable  
10 marker comprises a visible marker and the second visible or selectable marker comprises a selectable marker.

111. The vector of claim 103, wherein the visible marker is GFP and the selectable marker is neomycin resistance.

112. A vector comprising in the direction of transcription: (a) a  
15 polynucleotide comprising a transcription regulatory sequence; (b) a 5' splice donor site; (c) a first inverted repeat; (d) a 3' splice acceptor site; (e) a polynucleotide encoding a transposase; (f) a polynucleotide encoding a selectable marker; and (g) a second inverted repeat.

113. The vector of claim 103, wherein the first and second inverted repeat  
20 comprises *Mariner* inverted repeats.

114. The vector of claim 103, wherein the transposase comprises a *Mariner* transposase.

115. The vector of claim 103, wherein the first and second inverted repeats comprise *Mariner* inverted repeats, and the transposase comprises a *Mariner*  
25 transposase.